Abstract

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Project Title: A Screen for Novel Antimicrobials

Abstract: DESCRIPTION (provided by applicant): The long-term goal of this project is to develop a novel method for antimicrobial drug discovery, based on a targeted search for prodrugs. The potential advantage of prodrugs is considerable - being converted inside a microbial cell into a non-specific active molecule, such compounds will have the capability to "sterilize" an infection, something that currently available antibiotics lack. There is a considerable unmet need for any type of novel antibiotics, due to the rise of multidrug resistant pathogens, and the threat of engineered multidrug resistant bioweapons. The unmet need for compounds that can act against slow-growing or dormant pathogens, and persisting biofilms, is even greater. The rationale of the method is to screen for compounds against strains differentially expressing an essential enzyme potentially capable of converting a prodrug into an antimicrobial. A strain overproducing a converting enzyme will be more susceptible to a prodrug. A combination of genomics with HTP screening makes this straightforward method realistic. Genomics provides us with a list of good candidate enzymes that can potentially convert prodrugs into drugs, and a rational screening design will enable efficient identification and validation of hits. We validated this approach with E. coli overexpressing NfnB, the converting enzyme for metronidazole. The overexpression strain showed >100 fold greater sensitivity as compared to the wild type control (Preliminary Studies). Importantly, the population was completely sterilized, the first observation of this kind with an antibiotic. Lead compounds resulting from this screen will sterilize an infection, and since the converting enzyme is an essential protein, the probability of resistant mutations will be low, limited to rare modifications of the enzyme that specifically exclude the prodrug without affecting the natural substrate. We expect several outcomes from this project, both short- and long-term: (1) The proposed screen will provide proof-of-principle for a targeted search for prodrugs. (2) Validated hits we identify will become leads for antimicrobial drug development. (3) The prodrug screen will be applied to yeast in search of antifungal compounds. (4) A direct prodrug screen for anticancer agents using mammalian cells can be developed as well, based on the same general design that we will use in this project. Specific Aims: 1. A compound library will be screened to identify prodrug hits. This will be achieved in two steps: a. The original library of 500,000 compounds will be screened for direct activity against E. coli. This will be performed in a standard HTP format, detecting inhibition of growth activity by changes in OD. This will produce an active sub-library of ~10,000 compounds. b. Prodrug candidates will be identified from the sub-library by a screen against 50 individual E. coli strains each overexpressing a gene coding for a particular essential enzyme. 2. Validated prodrug candidates will be identified in a secondary screen. Hits will be tested against strains with a decreased expression of enzyme of interest. The activity of a hit compound in such a cell will be decreased, confirming its prodrug nature.

Thesaurus Terms:
High throughput screening, Antimicrobials, genomics, E. coli, NfnB, prodrug, antifungal compounds

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